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# Estimating Taxonomic Diversity Using Centrum Growth Profiles and Stinger Morphology of 36 Million Year Old Stingrays From North Dakota

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COLUMBUS STATE UNIVERSITY

# ESTIMATING TAXONOMIC DIVERSITY USING CENTRUM GROWTH PROFILES AND STINGER MORPHOLOGY OF 36 MILLION YEAR OLD STINGRAYS FROM NORTH DAKOTA

A THESIS SUBMITTED TO THE HONORS COLLEGE IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR HONORS IN THE DEGREE OF

BACHELOR OF SCIENCE

DEPARTMENT OF BIOLOGY

COLLEGE OF LETTERS AND SCIENCES

BY

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Approved by

Dr. Michael Newbrey, Committee Chair

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## ABSTRACT

Stingrays are a diverse and popular group of vertebrates; however, nothing is known about the relationships between growth biology and climate change. Freshwater stingrays once inhabit the United States and Canada during very warm times in the geologic record. No stingray material has been recorded from the northern part of the United States for the last 33 million years. The Earth's climate cooled from 50 to 33 million year ago when many warm adapted organisms were relegated to warmer, southern latitudes in North America. Today, freshwater stingrays only inhabit subtropical and tropical environments. We are interested in the freshwater stingrays that lived just prior to the climatic cooling that changed the area of what is now North Dakota 33 million years ago.

Our goal was to estimate alpha diversity of stingrays from a fluvial fossil deposit that existed 34 million years ago. We predicted the fossil stingrays from North Dakota to grow very slow compared to their modern counterparts. Fossil elements of stingrays are represented by their individual vertebral centra, stingers, and teeth. No complete specimens have been identified. We examined 36 isolated vertebral centra and estimated the number of growth cessation marks on each centrum. Centrum radial distance (mm) was measured from the notochord foramen to each annulus and plotted. We could not find any published data on age and growth of extant freshwater stingrays for comparison. The growth profiles were compared to marine *Dasyatis pastinaca*, Common Stingray, which are found in Northeastern Atlantic Ocean. There is complete overlap in the von Bertalanffy growth curves and parameters of the two datasets with no evidence for slow growth rates from ages 1-7 years old. Stingers were described morphologically and stinger thickness and median ridge thickness (mm) was measured for each specimen. Chronological ages for individual specimens ranged from 0 to 8 years old with two significantly distinct growth profiles; small and large profiles. Three stinger morphotypes were recognized. Measurements of stinger median ridge thickness indicated there were two small morphotypes and one significantly larger morphotype.

Our data suggest there were three taxa of stingrays that lived in the river channels of North Dakota 36 million years ago. Two taxa were small and one taxon was somewhat larger. Our next goal is to determine whether there are three distinct morphologies in the vertebral centra. There is little evidence of old individuals in the fossil dataset suggesting two hypotheses; 1) older individuals did not exist in the population, and 2) older individuals lived in another habitat (habitat partitioning). The use of fossils stands to provide great insight into the effects of climate change on the age and growth biology of fishes. Our research indicates that diversity of freshwater stingrays was higher than expected. Climatic cooling may have caused regional extinctions of freshwater stingrays because freshwater stingrays are found in tropical and subtropical areas today.

# ACKNOWLEDGMENTS

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# INTRODUCTION

Stingrays are an ancient and fascinating group of cartilaginous fishes captivating the attention of biologists and the general public because of their unique morphology and presence of poisonous stingers. Stingrays are known from the fossil record since the Early Cretaceous period (Cappetta 2012, Nelson et al., 2016). Stingrays are characterized by having a dorsoventrally compressed body with a large pectoral disc (Nelson et al., 2016). Most stingrays are marine, but two families exhibit examples of freshwater taxa, *Dayatidae* (whiptail stingrays) and the *Potamotrygonidae* (river stingrays) (Last et al., 2016). Dasyatidae contains several genera of tropical to warm-temperate rivers and lakes including *Dasyatis*, *Himantura*, and *Pastinachus sephen* (Nelson et al., 2016). Within the river stingray family, *Potamotrygonidae*, are four genera with 26 species from South America in the Atlantic and Caribbean drainages (Nelson et al., 2016). The freshwater forms are adapted to fresh water with a reduced rectal gland and low urea concentrations (Nelson et al., 2016).

The fossil record also shows evidence of freshwater stingrays in modern day Montana, Wyoming, and Utah. In Montana, fossilized teeth from the *Dasyatis* genus were found in the Tullock member of the Fort Union Formation and were aged to be 65 million years old (Cook et al., 2014b). However, no stingers or vertebrae have been described from the Fort Union Formation. The species *Asterotrygon maloneyi* and *Helophatis radians* were aged to be 50 million years old and present in the Green River Formation in Wyoming (Carvalho et al., 2004). Specimens of *Asterotrygon* and *Heliobatis* are known from complete skeletons (Carvalho et al., 2004). Teeth from the species *Saltirius utahensis* were recovered from the freshwater Brian Head Formation in Utah and were aged to be 44 million years old (Cook et al., 2014a). The tooth morphology of this species resembled that of the species *Asterotrygon maloneyi*, a specimen that was included in Carvalho et al.'s (2004) research of freshwater stingrays in the Green River Formation in Wyoming (Cook et al., 2014a). However, no stingers or vertebrae have been described from the Brian Head Formation.

Fishes are ectotherms meaning that they cannot control their own body temperature. Temperature, therefore, has a great impact on their growth, migration, and spawning (Cook et al., 2014). Cook also hypothesized that the stingrays disappeared from the fossil record beginning during the late Eocene Epoch (33 million years ago) when the Earth experienced a dramatic cooling. The early Eocene is characterized by having the highest mean annual temperatures of the entire Cenozoic Era with temperatures of roughly 30°C. The middle Eocene experienced a significant plate tectonics separating Antarctica and Australia. This created the circum- Antarctic Current, which changed the oceanic circulation patterns and global heat transport, resulting in a global cooling event observed at the end of the Eocene (Polly, 2009).

Regarding modern stingrays, very little is known about the age and growth of small marine or freshwater stingrays while nothing is known about age and growth of ancient stingrays. *Dasyatis pastinaca* (Linnaeus, 1758), otherwise known as the Common Stingray, is a marine batoid that is predominately found in the North-eastern Atlantic Ocean, the Mediterranean Sea and the African coast southwards of Senegal (Séret, 2003). This particular species can grow up to 60 cm in disc width (DW) and 250 cm in total length (IBSS, 2018; Ferretti et al., 2005). The maximum recorded age of *D. pastinaca* is 10 years, but captive animals of up to 21 years old have been reported (Ismen, 2003). Recently, we collected 37 individual vertebral centra of an unidentified species of stingray from a fossil locality in North Dakota.

These organisms were present in this location before the Earth experienced a dramatic cooling period approximately 33 million years ago during the late Eocene Epoch. The goal of my research was to describe centrum and stinger morphologies and analyze the age and growth rates of the fossilized specimens. I hypothesized that diversity would be low and that the fossilized stingray found in the freshwater locality would have a similar growth pattern to the modern saltwater species of *Dasyatis pastinaca.* However, their maximum size is hypothesized to be smaller with a shorter lifespan in the cooler climate of North Dakota.

## MATERIALS AND METHODS

### Background on stingrays

*Dasyatis pastinaca* (Linnaeus, 1758), otherwise known as the Common Stingray, is a marine batoid that is predominately found in the North-eastern Atlantic Ocean, the Mediterranean Sea and the African coast southwards to Senegal (Séret, 2003). *Dasyatis pastinaca* inhabits marine coastal lagoons, shallow bays, and estuaries with murky bottoms. The species typically feeds on benthic invertebrates (Last et al., 2016).

There is one taxon of whiptail stingray in North America, *Hypanus sabinus* (Lesueur, 1824) that inhabits freshwater (Robins et al., 2018). Their distribution extends from the Gulf of Mexico (Mexico) north to Delaware (Last et al., 2016). Young are born live with litters of 2-3 pups and gestation periods of four months (Last et al., 2016). Adults are found in estuaries, over sandy substrates in large rivers, and often buried in soft sediment (Robins et al., 2018). Disc width (DW) is generally 20-25 cm in females and males but can be up to 60 cm DW in Florida (Last et al., 2016, Robins et al., 2018).

The physical description of stingrays includes, but is not limited to, a snout that uses chemoreceptors to locate food, eyes on the dorsal side of the animal with the mouth and gills slits on the ventral side, pectoral fins used for movement, and pelvic fins that can be used for reproduction (Nelson et al., 2016). Stingrays draw in oxygenated water through spiracles, located anterior to the eyes, and move the water across the gills. Stingrays defend themselves with stingers on a whip-like tail. Stingers are composed of calcium carbonate surrounded by tissues that release a toxin when impaling another organism (Carvalho et al., 2004).

**Location and collection**

Isolated centra of an unknown species (n = 37) of stingray were found with teeth and stingers while screen washing fluvial sediment from the Medicine Pole Hills locality, in the Chadron Formation, Eocene (36 million years old), North Dakota. The Medicine Pole Hill locality is Chadronian 2 in land mammals ages (36.6 mya to 35.8 mya) according to Dr. Clint Boyd, Heritage Center, Bismarck, North Dakota. The fossils were collected by Dr. Clint Boyd.

**Morphological terminology and description**

Terminology used to describe vertebral centrum morphology was taken from Ridewood (1921), Kozuch and Fitzgerald (1989), and Newbrey et al. (2015). Terminology used to describe caudal stingers was taken from Carvalho et al. (2004).

Ammonium chloride sublimate was used to enhance the surficial features of the centra. In addition to the use of ammonium chloride in visual enhancement, a scanning electron microscope was used to photograph vertebral centra and stingers.

The lateral surfaces of the stingers were also characterized as either having no serrations, coarse serrations, or fine, irregular serrations. I collected measurements of stinger thickness (mm) and median ridge width (mm) from each specimen. A ratio was used to examine the relative size distribution among the various morphotypes. The ratio was determined by dividing the ridge width (mm) by the stinger thickness (mm).

**Age and growth**

The chronological age (years) was recorded from the number of growth cessation marks found on each centrum. Growth cessation marks were distinguished as pairs of light and dark rings. Each pair of rings occurred with a concentric ridge on the surface of the corpus calcareum. The centrum radial distance was measured in (mm) from the notochord foramen of the centrum to each growth cessation mark to construct and plot a growth profile (Fig. 1).

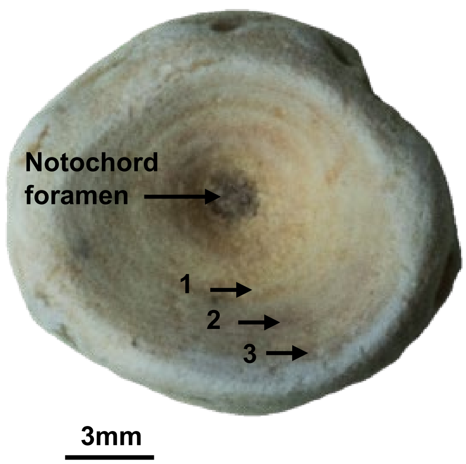



**Figure 1. Vertebral centrum from a 36 million year old freshwater stingray, Chadron Formation, North Dakota (ND 89002-E). The centrum was dusted with ammonium chloride sublimate to enhance the surficial features.**

Age and growth was examined using a von Bertalanffy (1938) growth curve:

$$CRD_t = CRD_\infty(1\text{-}e^{(-k(t\text{-}t_0))})$$

where the parameters: CRDt = centrum radial distance (mm), at t (age assumed to be in years); $CRD_\infty$ = theoretical maximum RD (mm); k = the Brody growth coefficient; t = time (i.e., age in years); and $t_0$ = time at age zero (time at theoretical zero length) in SYSTAT (Systat 12 2007). Size distributions were compared using a t-test with significance level set at $\alpha = 0.05$.

I compared chronological age and growth of the fossil material to published data from the marine stingrays *Dasyatis pastinaca* and *Hypanus americanus*. and acquired Çiğdem Yiğnin and Işmen's (2012) age and growth dataset of *Dasyatis pastinaca* for comparison to the fossil material. Age and centrum radial distance (mm) was compared among taxa. Çiğdem Yiğnin and Işmen (2012) described the age and growth for *Dasyatis pastinaca*. They found this particular species can grow up to 60 cm in disc width (DW) and 250 cm in total length (TL). The range of centrum radial distance for females was 1.23 – 5.35 mm, the total length ranged from 37.5 – 112 mm, while the age ranged from 3 – 16 years old. The range of centrum radial distance for males was 1.41 – 2.84 mm, the total length ranged from 40-80 mm, while the age ranged from 4 – 10 years old.

## RESULTS

Of the 37 vertebral centra that were collected, each centra was placed into one of four categories based on morphology (Fig. 2). Centrum type 'large A' exhibit characteristically elongate fossae, but type 'large B' had multiple smaller fossae. Centrum type 'small A' exhibit large square-shaped fossae, and 'small B' are characteristically short centra that have circular and multiple fossae.

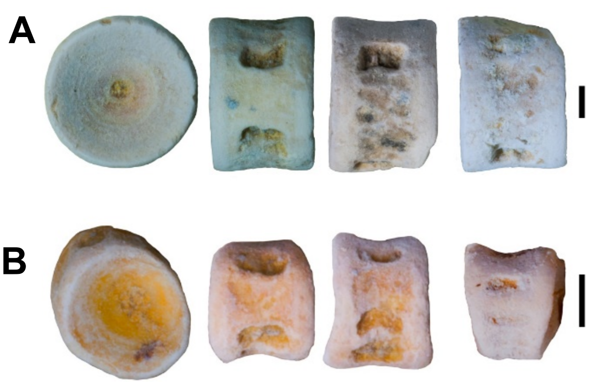



Figure. 2. Two centrum morphotypes from 36 million year old freshwater stingrays from North Dakota. **A**, large morphotype NDGS 3523. **B**, small morphotype NDGS 3534.Centrum views: anterior, left lateral, dorsal, and ventral. Scale bars = 1 mm.

The fossil stingrays from North Dakota ranged from 0 to 5 years old (Fig. 3A). Centrum radial distances at birth ranged from 0.11 to 0.36 mm. Maximum centrum radial distance was 1.76 mm. A plot of the growth profiles reveals two significantly different size distributions (t-test: N = 24, P < 0.001); small and large sets of growth profiles. At age 1, centrum radial distances ranged from 0.28 to 0.46 mm for the small growth profile (N = 64). At the same age, centrum radial distances of the large growth profile ranged from 0.52 to 0.69 mm (N = 106).

We modeled the small and large growth profiles with a von Bertalanffy growth model (Fig. 3B). The small von Bertalanffy growth profile is straight in shape with a maximum age of 5 years old and a maximum recorded centrum radial distance of 0.92 mm ($R^2$ = 0.942, $CRD_\infty$ = 2.500 [95% CI = 0.869–4.131], K = 0.104 [95% CI = 0.017–0.192], $t_0$ = -0.593 [-0.795– -0.392]). The large von Bertalanffy growth profile (N=106) is slightly curvilinear with a maximum age of 8 years old and a maximum recorded centrum radial distance of 1.76 mm ($R^2$ = 0.950, $CRD_\infty$ = 4.000 [95% CI = 2.672–5.328], K = 0.098 [95% CI = 0.054–0.142], $t_0$ = -0.700 [-0.877– -0.523]).

The birth ring sizes of the small centra morphotype (N=21) had a centrum radial distance that ranged from 0.110 to 0.230 mm where the mean was 0.159 mm with a standard error of 0.008 mm. The birth ring sizes of the large centra morphotype (N=27) had a centrum radial distance that ranged from 0.210 to 0.360 mm where the mean was 0.290 mm with a standard error of 0.008 mm. A two-sample t-test on CRD at birth showed that t = 11.980, df = 44.3, and $p < 0.001$.

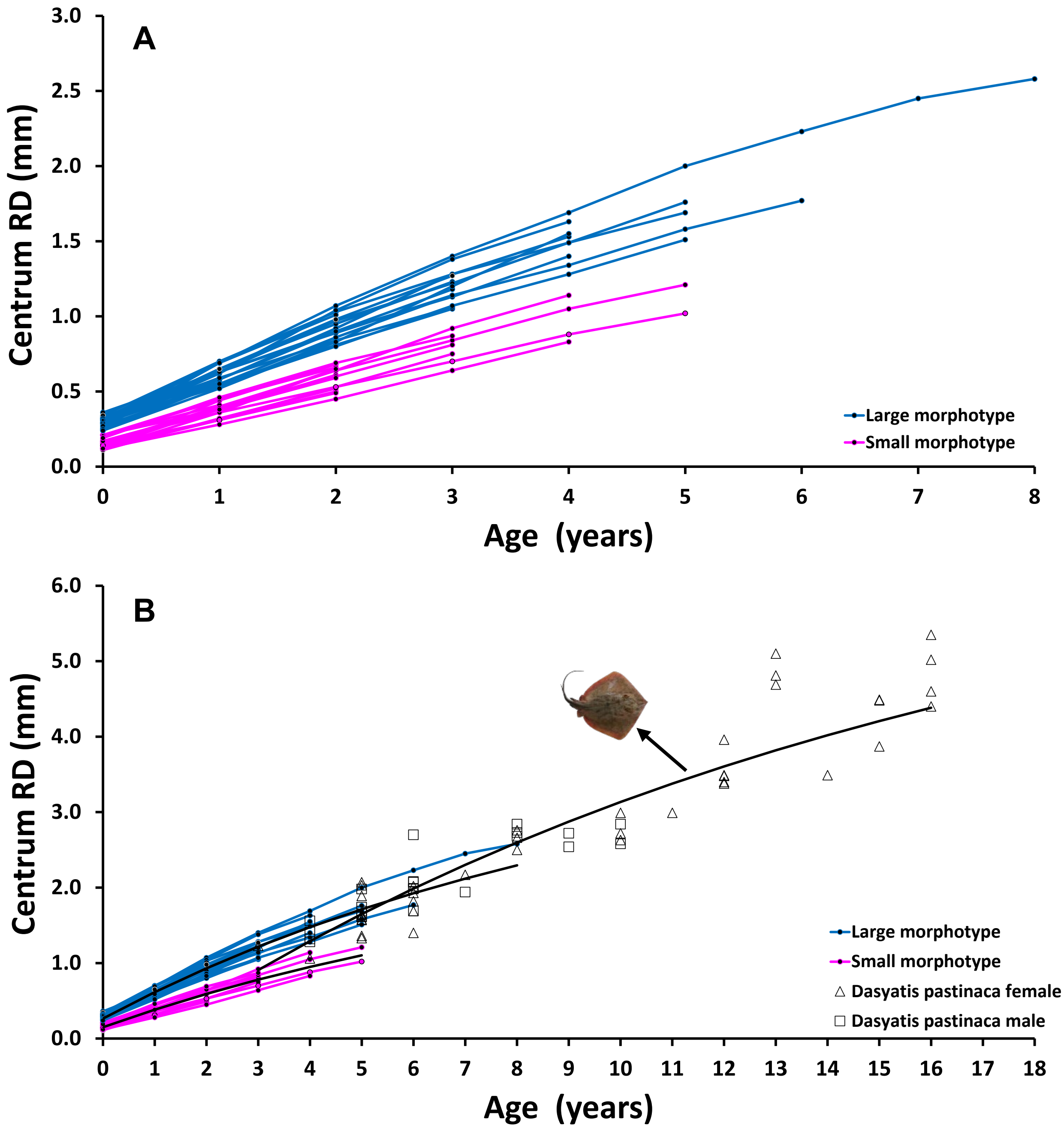



**Figure 3. Growth profiles of two centrum morphotypes from 36 million year old freshwater stingrays.** Each growth profile represents an individual centrum. Centrum radial distance is the measurement from the notochord foramen to each growth cessation mark. **A**, Large and small centrum morphotypes are depicted next to their respective growth profiles. **B**, Comparison of von Bertalanffy growth profiles among the two stingray morphotypes and *Dasyatis pastinaca* (males and females combined). Image from http://shark-references.com.

For CRD at age two between the small (N=14) and large centra morphotypes (N=20), we found that $t = 9.080$, $df = 31.9$, and $p < 0.001$. The centrum radial distances of the large fossil stingrays at age four, range in size from 1.28 to 1.69 mm. For comparison, the centrum radial distances of the extant *Dayatis pastinaca* (Cigdem Yignin and Ismen, 2012), at ages 4-5, range in size from 1.06 to 2.03 mm (N=62 with females and males combined). We also modeled the *Dasyatis pastinaca* with a von Bertalanffy growth model. The *Dasyatis pastinaca* von Bertalanffy growth profile is slightly curvilinear with a maximum age of 16 years old and a maximum recorded centrum radial distance of 5.35 mm ($R^2 = 0.903$, $CRD_{\infty} = 7.000$ [95% CI = 3.519–10.481], $K = 0.065$ [95% CI = 0.010–0.120], $t_0 = 0.866$ [-0.476– 2.208]).

For stingers, we found that we had three different morphotypes. Type A distal stingers were easily distinguishable due to their serrated edges made up of equidistantly spaced, low, right triangles (Fig. 4A-C). Type B proximal stingers were smooth on the lateral edges and the ventral surface (Fig. 4D-F). Type C proximal stingers had characteristically small equidistantly spaced tubercles on the lateral edges as well as longitudinal striations on the ventral surface of the median ridge (Fig. 4G-I). The three different morphotypes are grouped based on the ratio of ridge to stinger thickness as well as external morphology (Fig. 5). We found no significant difference between Types A and B in ridge width (mm)($f = 22.042$, $df = 2, 35$, $p < 0.001$; Fig. 6) and stinger thickness (mm)($f = 12.632$, $df = 2, 35$, $p < 0.001$; Fig. 7). Type C was significantly different from both Types A and B being notably larger in both instances.

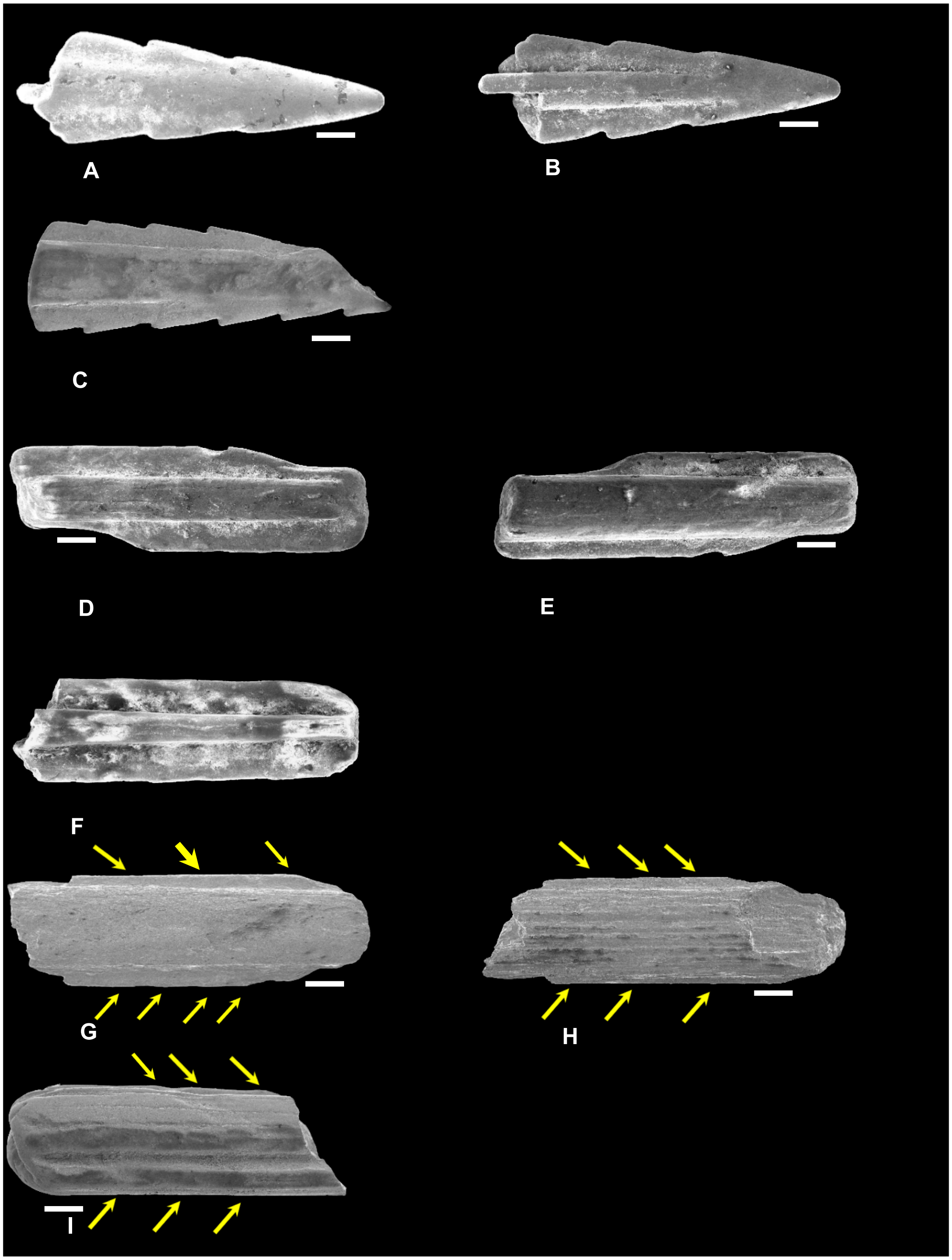



**Figure 4. Type, view, and specimen number of stingers found in Medicine Pole Hills locality, in the Chadron Formation, of North Dakota. (A** and **B)** Dorsal view of Type A specimen NDGS 3570 followed by its ventral view. (**C**) Ventral view of Type A specimen NDGS 3572. **(D** and **E)** Dorsal view of Type B specimen NDGS 3582 followed by its ventral view. (**F**) Ventral view of Type B specimen NDGS 3574. **(G** and **H)** Dorsal view of Type C specimen NDGS 3583 followed by its ventral view. (**I**) Ventral view of Type C specimen NDGS 3575.

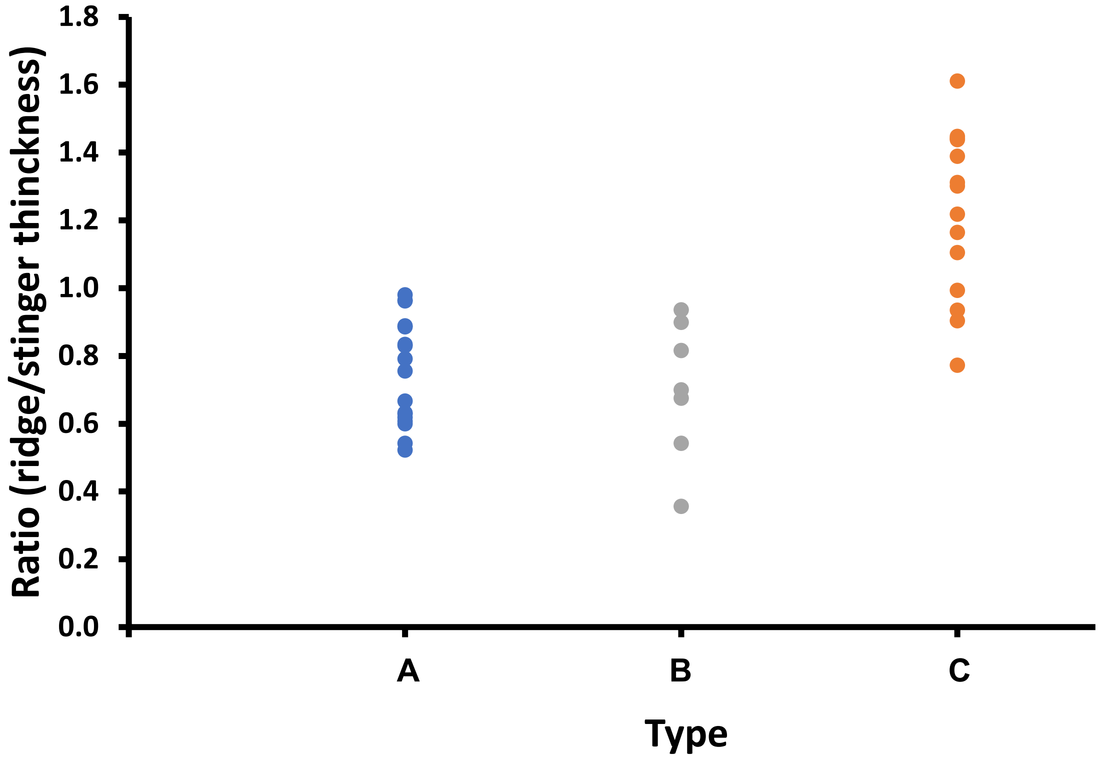



**Figure 5. Three morphotypes of stingers differentiated by ratio of ridge to stinger thickness.**

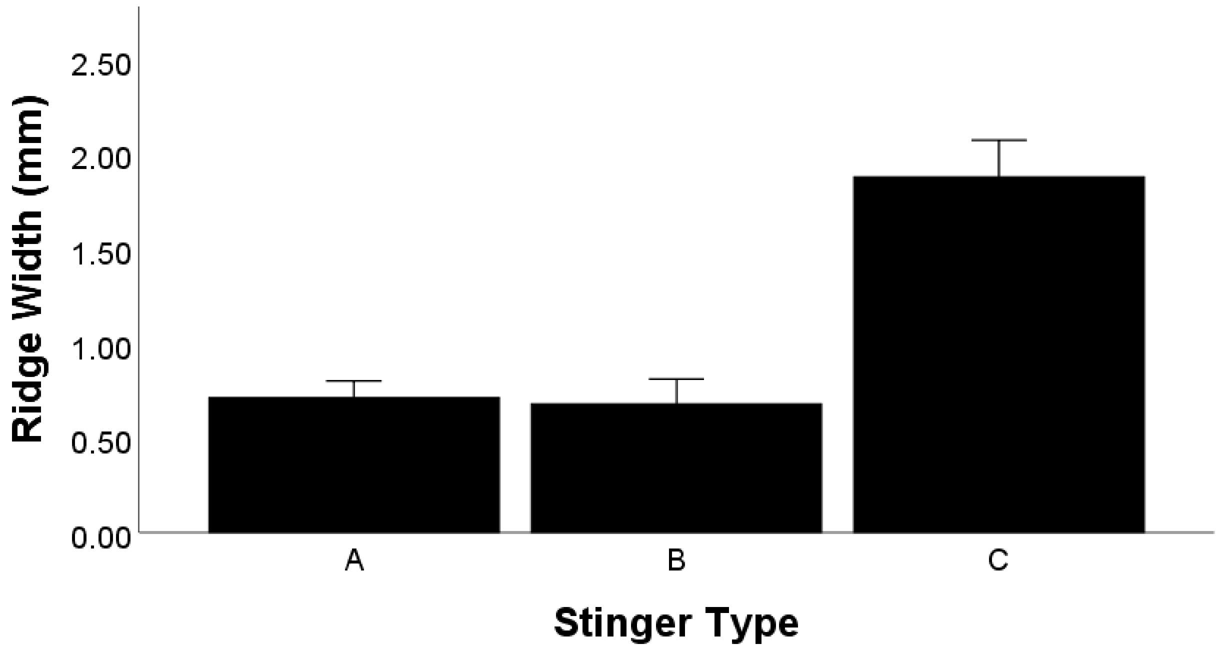
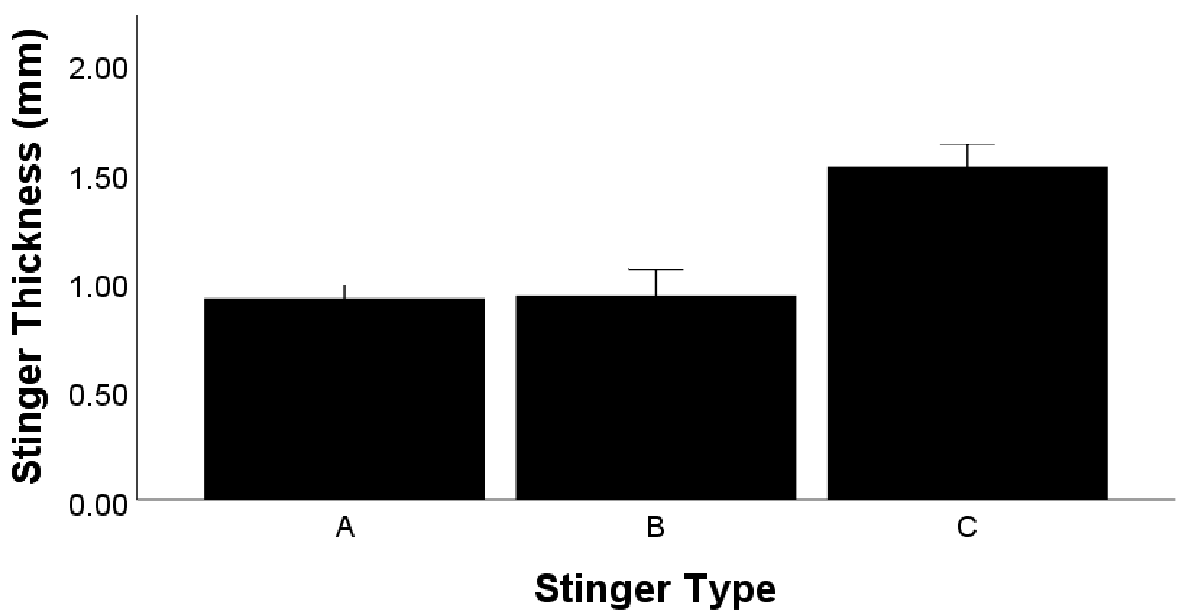



**Figure 6. Effect of stinger type on average (+/- 1 S.E.) ridge width.** Treatments sharing letters are not significantly different.



# DISCUSSION

I identified two distinct sets of stingray growth profiles from fossil centra and three distinct sets of morphotypes from the stingers. Of the small growth profiles, no individuals lived past five years in age. Individuals of the large growth profile did not live past eight years old. Older individuals were rare and larger in size than individuals of the previous age class. This suggests either 1) the largest and oldest individuals represented an age/size of mature adults or 2) that older/larger individuals lived elsewhere (habitat partitioning).

We prefer the former hypothesis because the fossil locality represents a pupping ground with individuals of age 0 represented in the sample. The rare, older individuals must represent breeding adults being 3-5 times larger than the pups.

This is not unlike the size range seen in other complete fossils of freshwater stingrays. For example, adults of *Asterotrygon maloneyi,* Green River Formation, Eocene, are 4.4 times the body length of their pups, and their centrum diameters are four times the size of those in their pups (Carvalho et al., 2004). There is a complete overlap in the centrum sizes of the extinct species of stingray from North Dakota and the extant species of marine *Dasyatis pastinaca* (Cigdem Yignin and Ismen, 2012). This overlap lends support for our age assignments in that the species have similar sizes at age.

Lifespans of the fossil stingrays were shorter than those of *Dasyatis pastinaca*; perhaps because the extinct stingrays lived during a time of dramatic cooling during the late Eocene epoch. Additional data is needed for comparison to examine this hypothesis. We found no published information concerning the age and growth patterns of freshwater stingrays.

The three different morphotypes of stingers supports the idea that there was a diversity of freshwater stingray found in the fluvial fossil deposit of North Dakota. This goes on to form the basis of my conjecture that, instead of the two taxa that I identified from growth profiles, there may be an additional taxon present based on stinger morphology. This leads me to suggest that the morphology of stingrays in North Dakota 36 million years ago was more diverse than modern freshwater stingrays in North America.

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